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The Use of Ferrokinetics in the Study of Experimental Anemia

by Eun Woo Lee,* James J. Kocsis,† and Robert Snyder†

Erythropoietic cells in bone marrow are vulnerable to cytotoxic substances. There are three types of erythroid precursors: cells that can take up Fe but do not proliferate (reticulocytes), those that can take up Fe and proliferate (normoblasts and pronormoblasts), and those cells that do not take up Fe but can proliferate and differentiate into the erythroid cell line (ERC and stem cells). Each of these erythroid precursors requires a certain time before they emerge into the peripheral blood as mature red blood cells. By applying our understanding of ferrokinetics associated with erythropoiesis, it was possible to estimate a cytotoxic effect of chemicals on proliferating erythroid precursors (pronormoblasts) in mice by measuring 24-hr ^{59}Fe uptake in red blood cells 48 hr after treatment with chemicals. The effect of chemicals on pluripotent hemopoietic stem cells in mice was also estimated by measuring 24-hr ^{59}Fe uptake 72 hr after treatment with chemicals. The validity of experimental schemes was tested using cytarabine, methotrexate, vinblastine, cyclophosphamide, and busulfan, which are known to act against specific cell types. Effects on pluripotent hemopoietic stem cells were tested with or without activation of stem cells in G_0 into cell cycle. Applications of the ^{59}Fe uptake method in the study of (1) benzene toxicity and (2) effect of pentobarbital on the toxic action of hydroxyurea and cytarabine are described. Proper application of the ferrokinetic characteristics of erythropoietic cells enables the establishment of a methodology which can be used to evaluate potential toxic effects of chemicals on erythroid precursor cells and pluripotent hemopoietic stem cells.

Introduction

Bone marrow, a dynamic system which produces blood cells, is vulnerable to cytotoxic agents. The erythropoietic system retains several characteristics that allow for a rather precise experimental evaluation of the system: the response of early erythroid precursor cells to erythropoietin, the capability of erythroid cells to synthesize hemoglobin, and the existance of a histochemical marker for reticulocytes. By using ferrokinetic features and other developmental characteristics of cells involved in erythropoiesis, it was possible to measure the relative size of a cellular pool of different erythroid precursor cells affected by chemical agents. We believe such a methodology can be used for an assessment of hazard caused by cytotoxic agents on erythropoietic cells including hemopoietic stem cells.

This presentation will discuss: ferrokinetics involved in erythropoiesis, rationale in the application of ferrokinetics for evaluating chemically induced erythropoietic toxicity, an experimental scheme and test for the rationale, and application of the ^{59}Fe uptake method for the assessment of potential erythropoietic toxins.

*Biomedical Science Department, General Motors Research Laboratories, Warren, Michigan 48090.

†Department of Pharmacology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107.

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Erythropoiesis

The earliest erythropoietic cell that can be recognized morphologically is pronormoblast. This cell is derived from the erythropoietin response cell (ERC), and the ERC is derived from pluripotent hemopoietic stem cells. There is evidence that pluripotent hemopoietic stem cells are the progeny of more primitive hemopoietic stem cells (*1-4*). Pronormoblasts differentiate and mature sequentially into normoblasts, reticulocytes, and erythrocytes (Fig. 1). All cells from the ERC to the normoblast are in cell cycle and thus proliferate (*5, 6*) while reticulocytes and erythrocytes do not. In rodents, most pluripotent hemopoietic stem cells (90-95%) are not in cycle; i.e., they are in G_0 state (*7, 8*), but they can be activated into cell-cycle upon specific physiological stimuli (*9-11*). Under normal conditions, only 5-10% of stem cells are in cell cycle, and these constitute the ERC pool. The cells from pronormoblasts to reticulocytes can synthesize hemoglobin (*6*). Therefore, they can take up ^{59}Fe, but stem cells and ERC do not take up Fe because they do not make hemoglobin. Thus, from the ferrokinetic point of view, the cells involved in erythropoiesis can be classified into three categories: cells that take up Fe but do not proliferate (reticulocytes), cells that take up Fe and proliferate (normoblasts and pronormoblasts), and finally, cells that do not take up Fe but can proliferate and differentiate into the erythroid cell line, i.e., ERC and stem cells.

Ferrokinetics for Evaluating Chemically Induced Erythropoietic Toxicity

The time required for each of the erythrocyte precursor cells to mature and to be released into the circulating blood has been determined in mice. The turnover time of reticulocytes and the generation times of both pronormoblasts and normoblasts are about 24 hr (*12-15*). In these studies, it was decided that the size of the reticulocyte pool would be used as a measure of the effects of chemicals on each of the earlier cell types by administering ^{59}Fe, which rapidly disappears from the circulation, and sampling the peripheral blood for ^{59}Fe uptake 24 hr later, a time at which the label in the blood represents incorporation of the iron into hemoglobin during the period when maturation and release of the cells into the circulation occurs. Therefore, the cytotoxic effect of an agent on normoblasts in mice can be evaluated by giving ^{59}Fe when the affected erythroid precursors mature to the stage of reticulocytes, i.e., 24 hr after treatment with the agent and by measuring the amount of ^{59}Fe in the circulating red blood cells 24 hr after giving ^{59}Fe, a time interval which allows the bone marrow reticulocytes to be released into the blood (Fig. 1). The time when ^{59}Fe is injected as opposed to the time when the blood sample is taken is the time when the size of the reticulocyte compartment is measured in

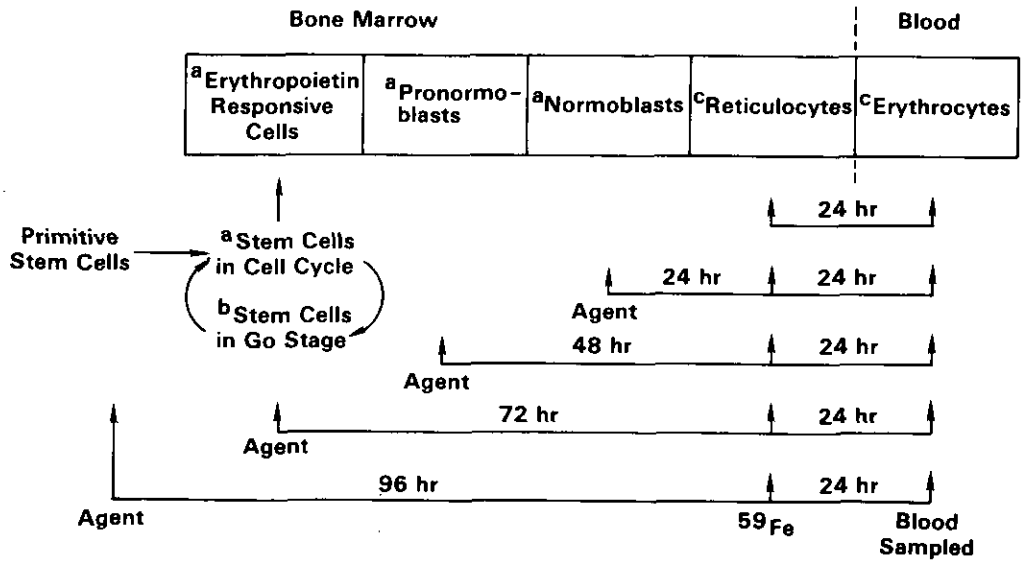

FIGURE 1. Developmental sequences of cells involved in erythropoiesis experimental scheme for determining the effect of agent on each cellular compartment: (*a*) proliferating cells; (*b*) nonproliferating cells but can be activated to proliferating cells; (*c*) nonproliferating mature cells.

cyt ^{59}Fe injection. By the same token, the effect of agents that damage the pronormoblasts can be reflected by the 24 hr ^{59}Fe uptake value which is measured 48 hr after treatment with the agent.

It has also been shown that it takes 24 hr for the ERC to differentiate into pronormoblasts (*12*). Regarding stem cells, there is no information on the time required for the cells to differentiate into ERC. However, our experimental data, which will be discussed shortly, suggest that stem cells differentiate into ERC immediately upon physiological stimulus. Thus, we suggest that the effect of agents that inhibit pluripotent hemopoietic stem cells and ERC can be seen when ^{59}Fe is given 72 hr after treatment with the agent and 24 hr ^{59}Fe uptake is measured.

Experimental Scheme and Tests

In order to test the validity of the rationale, three types of cytotoxic agents, for which the mechanism of action is known, were chosen. Methotrexate and cytarabine are S-phase specific cytotoxic agents and can kill only proliferating cells (*16-22*), while cyclophosphamide and busulfan are cell-cycle stage nonspecific agents and can kill both proliferating and nonproliferating cells such as hemopoietic stem cells (*23-26*). Vinblastine produces both metaphase arrest of mitosis and some damage to hemopoietic stem cells (*23, 27*). The effect of these cytotoxic agents on each compartment of erythroid precursors were determined by the 24-hr ^{59}Fe uptake measured 24, 48, 72, and 96 hr after injection of a single dose of the agent.

Cytarabine and methotrexate lowered ^{59}Fe uptake 24 and 48 hr after treatment with the agent, respectively, as expected, but at 72 and 96 hr ^{59}Fe uptake was increased over control values (Fig. 2). An interpretation of the overshoot may be that the ERC pool was immediately replaced by an excessive number of pluripotent stem cells as a compensatory mechanism when cytarabine and methotrexate killed the proliferating ERC, pronormoblasts, and normoblasts (*28*). An alternative explanation could be that more cell divisions might be inserted in the pronormoblast and normoblast compartments by a shortening of the intermitotic time (*29*). However, this latter process cannot be the reason for the overshoot because ^{59}Fe is given after the cells of the maturing compartments have already been released into the blood. Thus, the overshoot indicates that the ERC pool was immediately replaced by an excessive number of stem cells. should have been observed when it was measured 72 hr after treatment with methotrexate or cytarabine because they kill ERC. Cyclophosphamide and bulsulfan reduced ^{59}Fe uptake at all times, as expected, confirming that the action of cell-cycle stage nonspecific agents on nonproliferating stem cells can be measured by the ^{59}Fe uptake method. Vinblastine reduced the ^{59}Fe uptake at both 24 and 48 hr and also less severely at 72 hr, as expected. Here again, the ^{59}Fe uptake at 96 hr after vinblastine exceeded the control value, suggesting that the damage on erythropoietic system activated G_0 stem cells into cell cycle.

The fact that the 24-hr ^{59}Fe uptake measured 72 hr after treatment with cytotoxic agents reflects the effect of the agents on the stem cell compartment is further supported by the following experiments. Three doses of 50 mg/kg of cytarabine were given every 2 hr over a 4-hr period and 24-hr ^{59}Fe uptake was measured 72 hr after the last dose of cytarabine. Under these conditions, the ^{59}Fe uptake was not affected (Table 1). However, when a primary dose of 50 mg/kg of cytarabine was given 12 hr prior to the same series of three doses of 50 mg/kg at 2-hr intervals and 24-hr ^{59}Fe uptake was measured 72 hr after the last dose, a significant reduction of ^{59}Fe uptake by 65% was produced. On the basis of the spleen colony forming unit assay (CFU-S) (*30*), it has been shown that pluripotent stem cells in the G_0 state can be induced to synthesize DNA and divide by cytarabine (*10, 31*).

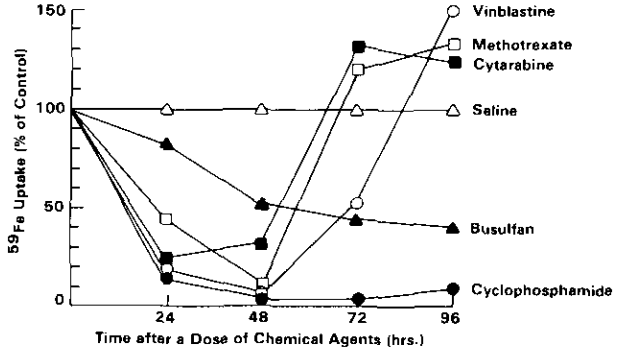

FIGURE 2. Twenty-four hour ^{59}Fe uptake in mice measured at different time intervals following a single IP dose of cytarabine (150 mg/kg), methotrexate (20 mg/kg), vinblastine (2 mg/kg), cyclophosphamide (200 mg/kg), and a single sc dose of busulfan (320 mg/kg). Values represent the mean of 10 mice per group and were significantly different in all cases except those at 24 hr after busulfan, 72 hr after methotrexate, and 96 hr after cytarabine; 72 hr after cytarabine and also at 96 hr after methotrexate and vinblastine the significance of the differences was $p < 0.05$ whereas in all other cases the differences were $p < 0.001$.

Thus, primary dose of cytarabine, but no reduction without the primary dose, further supports the notion that the 24-hr ^{59}Fe uptake measured 72 hr after treatment with cytotoxic agent reflects the effects of agents on nonproliferating pluripotent stem cells.

Application of the ^{59}Fe Uptake Method for the Assessment of Potential Erythropoietic Toxins

Erythropoietic Toxicity of Benzene Measured by the ^{59}Fe Uptake Method

Effect of a single subcutaneous dose of benzene (88, 440, and 2200 mg/kg as a 50% benzene-olive oil solution) in mice was evaluated by parameters such as erythrocyte ^{59}Fe uptake, total leukocyte counts and hematocrits 24 hr after benzene administration. To determine the ^{59}Fe uptake, mice were given ^{59}Fe (0.5 μCi, 20-40 ng of iron) as ferrous citrate 24 hr after benzene treatment and bled 24 hr later. Unless specified, ^{59}Fe was injected IP throughout the study. Blood (0.2 ml) from each mouse was counted in a scintillation well counter and the percentage of the ^{59}Fe taken up into erythrocytes was calculated, assuming a blood volume of 6% of the body weight. Statistical significance was determined by means of Student's *t* test. Experimental results in Table 2 indicate that the ^{59}Fe uptake measurement is a more sensitive method than total leukocyte counts and hematocrits for evaluating bone marrow activity.

The stage of erythroid cell development sensitive to benzene was evaluated using the ^{59}Fe uptake method. Mice were given single doses of benzene sc and its effect on ^{59}Fe uptake was evaluated after five specific time intervals (Fig. 3). No suppression was found after 1 and 12 hr and also 72 hr, whereas dose-dependent inhibition of the ^{59}Fe uptake was or 2200 mg/kg dose. Thus, the data can be interpreted to suggest that (1) benzene did not interfere with an incorporation of iron into heme, (2) benzene interfered with proliferation of normoblasts and pronormoblasts, and (3) benzene did not damage hemopoietic stem cells which were in the G_0 state at the time of benzene injection.

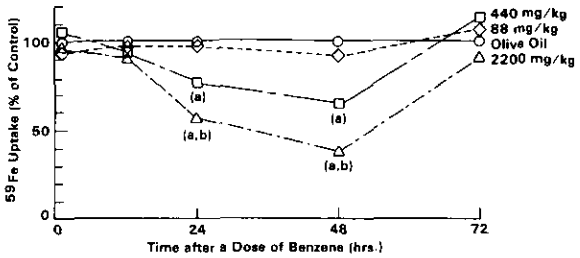

FIGURE 3. Effect of benzene on 24-hr erythrocyte utilization in mice measured at different time intervals following a single sc dose of benzene as a 50% (*v/v*) benzene-olive oil solution: 88, 400, and 2200 mg/kg. Control group received olive oil only. Values represent the mean of more than 11 mice per group (11 to 26); (*a*) significantly different from control ($p < 0.001$); (*b*) significantly different from 440 mg/kg dose ($p < 0.01$).

The effect of toluene on benzene toxicity and metabolism was also studied by using the ^{59}Fe uptake method. [^{3}H]Benzene was given subcutaneously (SC) at a dose of 440 or 880 mg/kg and the metabolites in urine were measured over a 24 hr period with or without toluene (1720 mg/kg, SC). At 48 hr after giving benzene, ^{59}Fe was administered to determine the degree of benzene toxicity. Table 3 shows that toluene reduced the appearance of benzene metabolites in the urine to 30-44% of controls. Toluene did not affect ^{59}Fe uptake but did alleviate the reduction in ^{59}Fe uptake caused by benzene. Taken together, these results suggest that inhibition of benzene metabolism was accompanied by alleviation or prevention of benzene toxicity and that benzene toxicity was probably

Table 1. Effect of dose schedules of cytarabine on ^{59}Fe uptake in mice.

Total dose of cytarabine, mg/kg	Schedule of administration	24 hr ^{59}Fe uptake measured 72 hr after cytarabine (mean ± SD), %[a]
Saline control	Three times, 0.2 ml every 2 hr	22.6 ± 7.6 (8)
150	Three times, 50 mg/kg every 2 hr	23.2 ± 8.0 (10)
300	Single injection	29.0 ± 10.6 (9)
200	Initial dose, 50 mg/kg 12 hr prior to three successive doses of 50 mg/kg every 2 hr	7.8 ± 6.1[b] (6)

[a]Number of animals given in parentheses.
[b]Significantly lower than control values ($p < 0.001$).

Benzene dose, mg/kg	24-hr ^{59}Fe utilization, %		Total leukocyte/mm^3		Hematocrit
	Mean ± SD[a]	CV[b]	Mean ± SD[a]	CV[b]	Mean ± SD[a]
Olive oil control	24.3 ± 4.8 (14)[b]	19.8	5991 ± 2492 (16)	42	46 ± 3.8 (16)
88	24.7 ± 6.1 (19)	28.7	5540 ± 2519 (15)	45	45 ± 6.7(15)
440	17.8 ± 3.7[c] (19)	20.8	4661 ± 1633 (14)	35	47 ± 2.6 (14)
2200	12.1 ± 4.5[c] (15)	37.2	4585 ± 1551 (17)	34	46 ± 3.9 (17)

[a]Number of animals given in parentheses.
[b]CV = coefficient of variation (standard deviation/mean × 100).
[c]Significantly lower than the value of control group ($p < 0.001$).

caused by a toxic metabolite of benzene. The implication of benzene metabolites in benzene toxicity is further described in review articles (*33*, *34*).

The liver is generally accepted as the major site of benzene metabolism. Thus, the role of the liver in producing benzene-induced bone marrow depression was investigated using partially hepatectomized rats. Partial hepatectomy (*35*) resulted in removal of 75 ± 8% (16 rats) of the liver. Sham operations were performed by making a middle incision reaching 1-1.5 cm posteriorly from the xiphoid process of the sternum, exposing the median and left lateral lobes of the liver, and then closing the area with no liver excision. A single dose of benzene (2200 mg/kg) was injected SC 8 hr after surgery. Generally, labeled [^{3}H]benzene (approximately 80,000 dpm/μmole) was administered to determine benzene metabolites in urine (36 hr cumulative urinary radioactivity). Total radioactivity in urine was reported as benzene equivalents in μmoles. To determine radioiron utilization, ^{59}Fe (1.0 μCi per rat) was injected IP 48 hr after benzene administration and sample blood was collected 24 hours after ^{59}Fe administration for analysis. The percentage of ^{59}Fe incorporated into erythrocytes was calculated assuming a blood volume of 6% of the body weight.

Sham-operated and partially hepatectomized control rats showed no difference in ^{59}Fe uptake, indicating that partial hepatectomy itself had no effect on ^{59}Fe uptake. Benzene reduced ^{59}Fe uptake to about half of the control values in sham-operated animals (Table 4). No reduction in ^{59}Fe uptake, however, was observed in the benzene-treated partially hepatectomized rats, showing that partial hepatectomy protected the rat against benzene-induced bone marrow depression. On the other hand, partial hepatectomy markedly reduced benzene metabolism, confirming the requirement for the production of a metabolite which mediates benzene toxicity.

Effect of Pentobarbital on the Action of Cytarabine and Hydroxyurea to Hemopoietic Cells Measured by the ^{59}Fe Uptake Method

Since it has been reported that some hypnotics protected hemopoietic toxicity caused by x-irradiation

Table 3. Effects of toluene on benzene metabolism and on ^{59}Fe uptake in the mouse.

Treatment	[^{3}H] Benzene metabolism/24 hr, % of administered dose (mean ± SD)[a]	24 hr ^{59}Fe Utilization, % of administered dose (mean ± SD)[a,b]
Control		24.2 ± 4.7 (27)
Toluene (1720 mg/kg)		23.3 ± 4.6 (25)
Benzene (440 mg/kg)	35.8 ± 1.1 (2)[c]	15.7 ± 4.8[d] (21)
Benzene and toluene	10.9 ± 7.5 (2)[c]	22.0 ± 5.9[e] (28)
Control		18.8 ± 6.2 (22)
Toluene (1720 mg/kg)		15.8 ± 5.5 (19)
Benzene (880 mg/kg)	22.6 ± 5.7 (17)	4.9 ± 3.4[d] (19)
Benzene and toluene	9.9 ± 3.9 (17)	9.9 ± 4.5[e] (17)

[a]Number of mice given in parentheses.
[b]^{59}Fe was injected 48 hr after benzene or toluene administration.
[c]Two groups of animals, three animals per group.
[d]Significantly different from both control and toluene groups ($p < 0.05$).
[e]Significantly different from group receiving benzene alone ($p < 0.05$).

injection of benzene.

	Sham operation[a]	Partial hepatectomy[a]
Erythrocyte ^{59}Fe utilization, %[b]		
Control (olive oil)	31.6 ± 13.3 (9)[b]	29.1 ± 6.5 (10)
Benzene (2200 mg/kg, SC)	15.4 ± 6.7 (10)[c]	36.4 ± 16.2 (10)
Urinary metabolites, benzene equivalent, μmol[d]		
[^{3}H] Benzene (2200 mg/kg, SC)	132.2 ± 52.2 (4)	40.5 ± 19.8 (5)[c]

[a]Mean ± SD: the number of animals is given in parentheses.
[b]Based on administered dose: 24 hr ^{59}Fe uptake was measured 48 hr after benzene administration.
[c]Significantly different from control sham-operated rats ($p < 0.01$) and from benzene-treated partially hepatectomized rats ($p < 0.01$).
[d]Measured as 36 hr cumulative urinary radioactivity.

Table 5. Effect of pentobarbital on the action of multiple doses of cytarabine on the ^{59}Fe uptake measured 48 hr after treatment.

	24 hr ^{59}Fe uptake at various doses of cytarabine (mean ± SD), %[a]		
Groups	25 mg/kg × 3[b]	20 mg/kg × 3[b]	100 mg/kg × 3[b]
Control, saline	20.5 ± 8.9 (16)	19.8 ± 3.6 (16)	21.1 ± 3.9 (19)
Pentobarbital[c]	20.5 ± 5.2 (18)	20.7 ± 5.2 (17)	20.7 ± 5.8 (13)
Cytarabine	13.2 ± 4.7 (16)	6.6 ± 4.0 (17)	3.1 ± 1.5 (18)
Pentobarbital[d] + cytarabine	16.1 ± 5.4 (22)	11.8 ± 4.0[e] (21)	6.9 ± 2.8[e] (17)

[a]Number of mice given in parentheses.
[b]Three doses were given at 2 hr intervals. The 24 hr ^{59}Fe uptake was measured 48 hr after the last dose of cytarabine.
[c]Two or three successive doses of 60 mg/kg to maintain anesthesia for 6 hr.
[d]Pentobarbital (60 mg/kg) was given 1 hr prior to cytarabine and anesthesia was maintained for 6 hr by 1 or 2 additional dose(s) of pentobarbital.
[e]Significantly higher than the value of cytarabine treated group ($p < 0.001$).

(*36-38*) and cytotoxic agents (*39*), the effect of pentobarbital on the cytotoxic action of cytarabine and hydroxyurea to erythrocytic progenitors (pronormoblasts) and hemopoietic stem cells in cell cycle were investigated using the ^{59}Fe uptake method.

Data in Table 5 show that pentobarbital alone had no effect on pronormoblasts, while cytarabine given in three doses of 25, 50, or 100 mg/kg every 2 hr produced dose-dependent damage on pronormoblasts. When pentobarbital was given 1 hr prior to cytarabine and anesthesia was maintained until 1 hr following the last dose of cytarabine, the inhibition of ^{59}Fe uptake by cytarabine was significantly reduced at 50 and 100 mg/kg × 3, indicating that pentobarbital alleviated the toxicity of cytarabine on proliferating erythroid cells. Similar results were also obtained with hydroxyurea (Table 6).

Experimental results of pentobarbital action on the proliferating hemopoietic stem cells challenged with hydroxyurea is shown in Table 7. Here also, pentobarbital alone had no effect on the stem cells.

Table 6. Effect of pentobarbital on the action of multiple doses of hydroxyurea on the ^{59}Fe uptake measured 48 hr after treatment.

Group	24 hr ^{59}Fe uptake (mean ± SD), %[a,b]
Control, saline	40.6 ± 9.0 (15)
Pentobarbital[c]	37.9 ± 9.5 (13)
Hydroxyurea[d]	
167 mg/kg × 3	12.6 ± 3.9 (12)
333 mg/kg × 3	6.5 ± 2.8 (10)
Pentobarbital + hydroxyurea[e]	
167 mg/kg × 3	23.3 ± 7.3[f] (16)
333 mg/kg × 3	17.5 ± 4.7[f] (15)

[a]^{59}Fe was given IV.
[b]Number of mice given in parentheses.
[c]Two or three successive doses of 60 mg/kg to maintain anesthesia for 6 hr.
[d]Three doses were given at 2 hr intervals. The ^{59}Fe uptake was measured 48 hr after the last dose of cytarabine.
[e]Pentobarbital (60 mg/kg) was given 30 min prior to hydroxyurea and anesthesia was maintained for 6 hr by 1 or 2 additional dose(s) of pentobarbital.
[f]Significantly higher than the value of hydroxyurea treated group ($p < 0.001$).

Group	Schedule of administration	24 hr ^{59}Fe uptake (mean ± SD) %[a,b]
Control	Saline, 0.1 m three times at 2 hr intervals	34.1 ± 6.4 (13)
Pentobarbital	Two or three doses of 60 mg/kg to keep anesthesia for 6 hr	38.5 ± 9.5 (9)
Hydroxyurea		
Schedule A	Three successive doses of 500 mg/kg at 2 hr intervals	35.9 ± 7.7 (9)
Schedule B	Initial dose (2000 mg/kg) 12 hr prior to three doses of 500 mg/kg every 2 hr	11.5 ± 5.2[c] (10)
Pentobarbital + hydroxyurea (schedule B)	Hydroxyurea given by schedule B and pentobarbital given 30 min prior to 3 doses of hydroxyurea for 6 hr	22.8 ± 5.4[d] (10)

[a]^{59}Fe was given IV
[b]Number of mice given in parentheses.
[c]Significantly lower than control values ($p < 0.001$).
[d]Significantly higher than the value of hydroxyurea treated (schedule B) group ($p < 0.001$).

Table 8. Effect of pentobarbital on the action of cytarabine on the ^{59}Fe uptake measured 72 hr after treatment.

Groups	Schedule of administration	24 hr ^{59}Fe uptake[a] Mean ± SD, %	% of control
	Saline, 0.1 ml three times at 2 hr intervals		
Control	Two or three successive doses of 60 mg/kg to	23.2 ± 6.8 (10)	100.0
Pentobarbital	maintain anesthesia for 6 hr	23.4 ± 4.4 (10)	100.9
Cytarabine	Three successive doses of 150 mg/kg every 2 hr		
Schedule A	Initial dose (150 mg/kg) 12 hr prior to three suc-	22.4 ± 7.2 (8)	96.6
Schedule B	cessive doses of 100 mg/kg every 2 hr	3.9 ± 3.8[c](10)	16.8
Pentobarbital[b] + cytarabine (schedule B)	Cytarabine given by schedule B and pentobarbital given 1 hr prior to three successive doses of cytarabine	15.4 ± 3.3[d](10)	66.4

[a]Number of mice given in parentheses.
[b]Pentobarbital anesthesia was maintained for 6 hr by two or three successive doses of 60 mg/kg.
[c]Significantly lower than control values ($p < 0.001$).
[d]Significantly higher than the value of cytarabine treated group ($p < 0.001$).

Hydroxyurea, which is also a S-phase specific agent (*16, 40, 41*), did not affect the ^{59}Fe uptake when the stem cells are in G_0. However, when an initial dose of hydroxyurea (2000 mg/kg) was given 12 hr prior to three successive doses of hydroxyurea (500 mg/kg every 2 hr, schedule A), the ^{59}Fe uptake value was reduced to 32% of the hydroxyurea treated group given schedule A alone. Several groups of investigators have independently shown that an initial dose of hydroxyurea activated G_0 stem cells into cell cycle on the basis of the CFU-S assay (*11, 42, 43*). Thus, the present data confirms that the S-phase specific agent, hydroxyurea, can only kill the proliferating cells whether they are stem cells or pronormoblasts but it can not kill cells in the G_0 state such as resting hemopoietic stem cells. When pentobarbital is given 30 min prior to the three successive doses of hydroxyurea following an activation of G_0 stem cells, the ^{59}Fe uptake value was increased twofold. This protective effect of pentobarbital on proliferating stem cells was also observed when they were insulted with cytarabine (Table 8). The mechanism by which pentobarbital protects proliferating hemopoietic cells from cytotoxic agents remains to be seen. In summary, the proper application of ferrokinetics and other developmental characteristics of erythropoietic cells enables the establishment of a methodology which can be used to estimate the potential toxic effect of chemicals on erythropoietic cells and pluripotent hemopoietic stem cells.

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